HAPLOID SELECTION FOR LOW TEMPERATURE TOLERANCE OF TOMATO POLLEN

DANIEL ZAMIR,[1] STEVEN D. TANKSLEY[2] AND RICHARD A. JONES

Department of Vegetable Crops, University of California, Davis, CA95616

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ABSTRACT

Pollen grains were harvested from an interspecific F_1 hybrid between the cultivated tomato, *Lycopersicon esculentum* Mill., and its wild relative *Lycopersicon hirsutum* Humb. & Bonpl., a low temperature tolerant accession originating from an altitude of 3200 m in the Peruvian Andes. The two species differ for electrophoretically-detectable loci that mark six (possibly seven) of the 12 tomato chromosomes. Isozyme analysis of the BC_1 populations derived from controlled pollinations at normal and low temperatures indicates a significant skewing of allelic frequencies favoring two independent chromosome segments of *L. hirsutum* at low temperatures. The results demonstrate that gametophytic selection for low temperature tolerance of tomato pollen is determined, at least in part, by genes expressed in the haploid pollen.

PLANTS alternate regularly between the sporophytic and gametophytic phases of their life cycles. During the evolution of higher plants the gametophytic phase was dramatically reduced both in physical dimensions and in the relative time it occupied in the life cycle. This may explain why genetic research has been primarily devoted to the prominent sporophytic phase. In a unique study, CLEGG, KAHLER and ALLARD (1978) examined an experimental barley population for selection at different stages of the life cycle. Estimates were obtained for viability (zygote to adult) and fertility (adult to zygote) components of selection associated with electrophoretically detectable allozymes. Under their experimental conditions, gene frequencies were effectively changed due to gametophytic and sporophytic selection.

While selection can be demonstrated at the gametophytic stage, a major question remains: Is the selected genetic variation a product of the sporophytic (diploid) or gametophytic (haploid) genomes? As indicated by MULCAHY (1979), if genes expressed in the sporophyte determine gametophytic behavior, the number of different types of gametes cannot exceed the number of sporophytes in the population. However, if gametophytes are genetically self-determined, the potential number of gamete genotypes can be several orders of magnitude larger, depending upon the degree of heterogeneity of the population.

[1] Present address: Department of Field and Vegetable Crops, Hebrew University of Jerusalem, Faculty of Agriculture, P.O. Box 12, Rehovot, 76-100 Israel.
[2] Present address: Department of Horticulture, New Mexico State University, Las Cruces, NM 88003.

Thus, the number of gamete genotypes produced by a plant heterozygous for n loci is 2^n (ALLARD 1960).

Some gametic attributes are determined by sporophytic influence exerted during gametogenesis. In the male gametophyte (pollen grains) the outer wall (exine) and its protein components are apparently produced by the diploid anther tissue (HESLOP-HARRISON, KNOX and HESLOP-HARRISON 1974). Gene products of the diploid phase also determine the sporophytic incompatibility response such that the phenotype of the pollen grains is specified by the parent plant (DE NETTANCOURT 1977).

In higher plants, expression of genes by pollen is well documented and recently was reviewed by HESLOP-HARRISON (1979). Gametophytic control has been revealed for the gametophytic incompatibility barrier (EAST and MANGELSDORF 1925), pollen dimension and composition (BRINK and MACGILLIVARY 1924; MANGELSDORF 1932), pollen tube growth rate (MANGELSDORF and JONES 1926; SARI-GORLA, OTTAVIANO and FAINI 1975), and specific proteins (MULCAHY, MULCAHY and ROBINSON 1979; SCHWARTZ 1971; WEEDEN and GOTTLIEB 1979). TANKSLEY, ZAMIR and RICK (1981) examined seven dimeric enzymes in tomato pollen and demonstrated post-meiotic expression for all seven loci. These genes were apparently transcribed *in situ* by the pollen genome.

Of particular interest to plant geneticists are cases where differential fertilization has been demonstrated for pollen derived from single heterozygous individuals (JONES 1928; KEMPTON 1936; KEDAR, RETING and KATAN 1967). The contribution of the diploid tissue during pollen formation is probably identical for all the pollen grains borne by a single plant. The aberrant Mendelian ratios detected for marker genes in progeny of heterozygous plants can only be attributed to different constitutions of the gametes.

Selection among pollen grains can occur at two distinct developmental stages: 1) pollen formation (prepollination)—meiosis, microsporogenesis and release of mature pollen; and 2) pollen function (postpollination)—germination, tube growth and fertilization. The first has often been referred to as meiotic drive (SANDLER and NOVITSKI 1957) and the latter, as certation (DARLINGTON and MATHER 1949). However, these terms have been used loosely, often to refer to the same phenomenon. For clarity we will use the descriptive terms pollen formation and pollen function.

Selection at stages of pollen formation was documented in cases of microspore abortion due to pollen-killer genes in Nicotiana (CAMERON and MOAV 1957), wheat (LOEGERING and SEARS 1963) and tomato (RICK 1966). In each of these cases the pollen-killing effect is expressed post-meiotically, resulting from an interaction between genes in the maternal tissue and the pollen genome.

Selection at stages of pollen function has been demonstrated in pollen competition experiments. By varying the number of pollen grains (LEWIS 1954; TER-AVANESIAN 1978) or the length of the stigma through which the pollen tube had to grow (MULCAHY and MULCAHY 1975; MULCAHY 1971, 1974), different conditions for pollen competition were created. These selection schemes resulted in significant modifications of certain physiological parameters in the progeny populations. Indeed, the results must reflect an underlying genetic basis that can only be explained by the occurrence of selection at stages of pollen function.

Recently we compared the fertilization ability of pollen grains from the cultivated tomato, *Lycopersicon esculentum,* and an accession of the related wild species *Lycopersicon hirsutum*, which originated from an altitude of 3200 m in the Peruvian Andes (ZAMIR, TANKSLEY and JONES 1981). Pollen mixtures of the two species were used to pollinate flowers on plants maintained at high and low temperature regimes. Pollen grains of the high altitude hirsutum ecotype were substantially more successful in effecting fertilization at low temperature. The hirsutum pollen has also shown a significantly higher percent of germination *in vitro* at 5° than pollen of the cultivated tomato. These results demonstrate that hirsutum pollen is better adapted to chilling stress, in concordance with previous studies that established adaptive responses of the *L. hirsutum* sporophyte to chilling stress (PAULL, PATTERSON and GRAHAM 1979).

The objective of the present study was to determine if the adaptive response of hirsutum gametes is due to preconditioning by diploid maternal tissues or to genes expressed in the haploid pollen grains.

MATERIALS AND METHODS

In this study, pollen was harvested from the interspecific F_1 hybrid between *L. esculentum* cv. T_3 and *L. hirsutum* (LA 1777). The two species differ for alleles at nine previously described enzyme loci, seven of which have been situated on the tomato linkage map (TANKSLEY and RICK 1980b. TANKSLEY and JONES 1981): *Prx-1* chromosome *1*, *Pgm-2* chromosome *4*, *Adh-2* and *Aps-1* chromosome *6* about 9 cM apart, *Got-2* chromosome *7*, *Aps-2* chromosome *8*, *Prx-4* chromosome *10*. *Pgi-1* and *Est-4*, which are approximately 16 map units apart, have not been assigned to a chromosome but assort independently of the other loci, thus covering an additional part of the genome. Pollen from the F_1 hybrid was used to pollinate flowers of a male-sterile tomato variety (ms 10^{35}) in two growth chambers: one chamber was set at 6 hr cycles of 24° (light: 40 $\mu Em^{-2}s^{-1}$), 19° (dark) and the other on identical time cycle and light conditions but maintained at 12° (light), 6° (dark). The relative humidity in both chambers was 75%. After 96 hr the stigmas were excised and plants returned to the greenhouse. This experiment was replicated on three separate occasions using different female parents and batches of F_1 pollen collected from three different hybrids. Mature fruits were harvested and seed counts were taken from 12 mature fruits in each treatment. The BC_1 seeds were then sown in wooden flats in the greenhouse for subsequent isozyme analysis of the resultant seedlings. Plants of the BC_1 generation were cultured for electrophoresis as previously described (TANKSLEY and RICK 1980a) but due to a contamination by a plant pathogen we were unsuccessful in attempts to obtain complete data for each individual plant, as reflected in Table 1. Starch gel electrophoresis, enzyme extraction and activity staining were as reported by TANKSLEY and RICK (1980b).

RESULTS

Pollen harvested from the interspecific F_1 hybrid was used in crosses at high and low temperature regimes. The mean number of seed per fruit was 17.3 at 24/19° and 3.3 at 12/6°. The difference between the treatments ($D = 14.0$ $Sd = 2.4$) suggests that the number of pollen grains which complete fertilization is reduced at lower temperatures.

TABLE 1

Effect of temperature on number and percent of L. hirsutum *alleles in BC_1 populations*

	Experiment I		Experiment II		Experiment III		Total 24–19°		Total 12–6°		D	Sd*
Locus	24–19°	12–6°	24–19°	12–6°	24–19°	12–6°		%(A)		%(B)	(B-A)	
Prx-1	29/48	19/36	—	—	—	—	29/48	60.4	19/36	52.8	7.6	10.9
Pgm-2	—	—	29/65	25/54	29/43	17/24	58/108	53.7	42/78	53.8	0.1	7.4
Adh-2	24/42	26/35	17/35	20/26	21/42	17/24	62/119	52.1	63/85	74.1	22.0†	6.6
Aps-1	—	—	14/37	15/28	21/43	14/25	35/80	43.8	29/53	54.7	10.9	8.8
Got-2	—	—	15/37	11/27	22/43	10/24	37/80	46.3	21/51	41.2	5.1	8.9
Aps-2	22/46	15/36	13/37	9/28	15/43	11/24	50/126	39.7	35/88	39.8	0.1	6.8
Prx-4	—	—	18/34	14/24	23/43	10/24	41/77	53.2	24/48	50.0	3.2	9.2
Pgi-1	16/48	25/36	29/65	41/54	12/43	18/24	57/156	36.5	84/114	73.7	37.2†	5.6
Est-4	13/42	21/35	—	—	18/43	17/24	31/85	36.5	38/59	64.4	27.9†	8.1

$$* \; Sd = \sqrt{\frac{A(1-A)}{n_1} + \frac{B(1-B)}{n_2}}$$

Where n^1 and n^2 are respective sample sizes for the estimates at different temperatures and A, B are expressed as fractions.

† Significant at 0.001 level.

The frequency of *L. hirsutum* alleles in BC_1 seedling populations derived from crosses to the male sterile tomato variety under the two temperature regimes was determined (Table 1). The experiment was repeated on three separate occasions and the isozyme data was subjected to a heterogeneity Chi-square test. The results indicate that except for *Pgm-2* at normal temperature ($x^2 = 4.6$, 1 d.f., significant at the 5% level) all the observed frequencies could have been drawn from the same population and therefore the data was pooled. For the enzymic genes which mark portions of chromosomes *1*, *4*, *7*, *8* and *10*, no significant difference was observed for the frequency of the hirsutum alleles at the temperature treatments. The largest skewing in gene frequencies was observed for the marker gene *Pgi-1* (Figure 1). In pollinations at 24/19°, 36.5% of the progenies carried the hirsutum allele of *Pgi-1* whereas at 12/6° the frequency of this allele was doubled. *Est-4*, which is linked to *Pgi-1*, deviated to a lesser yet highly significant degree. The results demonstrate that a gene(s) linked to the marker loci, *Pgi-1* and *Est-4* affect fertilization ability of pollen at low temperatures; but we cannot rule out the possibility that the enzymic genes are the target of selection. Skewing was also observed for the marker gene *Adh-2* which is located on chromosome *6*. In pollinations at 24/19°, the frequency of BC_1 seedlings containing the hirsutum allele *Adh-2*^{1} was 52.1% and at 12/6°, 74.1%, a frequency very similar to the one obtained for the *Pgi-1*^{1} allele in the cold.

Since pollen grains of the genotype *Pgi-1*^{1} and *Adh-2*^{1} were favored during reproduction at 12/6°, the possibility exists for some type of genetic interaction between these loci in determining the cold temperature response. To test this, Chi-square for independence was calculated for the combined BC_1 data for these two alleles at 24/19° and 12/6° (Table 2). As reported in MATERIALS AND METHODS, we were not successful in our efforts to complete isozyme data for all the BC_1 generation; hence, only progeny for which information was available on both alleles were used in the analysis. At normal temperatures no evidence for interaction was revealed; at low temperatures a significant antagonis-

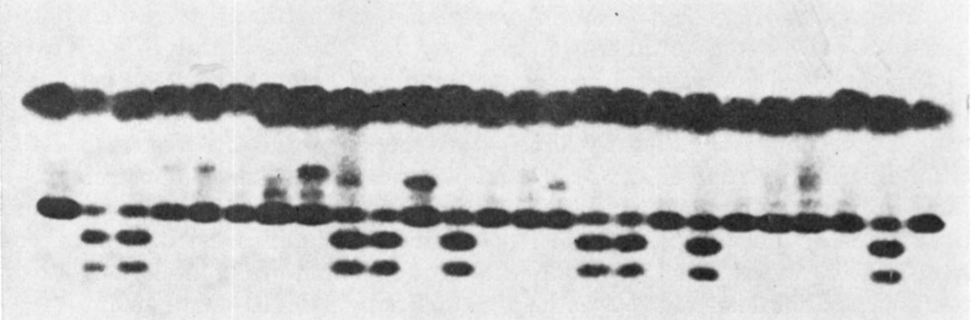

FIGURE 1.—A gel showing BC_1 segregation for *Pgi-1*. Each lane represents an extract from a single plant. The lanes with 3 dark bands are heterozygotes and those with one band are homozygotes.

TABLE 2

Interaction analysis for the Pgi-1 *and* Adh-2 *alleles in* BC_1 *progenies from crosses at 24/19° and 12/6°*

	24/19°				12/6°		
	Pgi-1				*Pgi-1*		
	+/+*	+/1*	Total		+/+	+/1	Total
+/+	25	13	38	+/+	2	23	25
Adh-2				*Adh-2*			
+/1	33	9	42	+/1	19	31	50
Total	58	22	80	Total	21	54	75

χ^2 (for independence) = 1.06 n.s. χ^2 (for independence) = 6.03 significant at 5% level.

* +, represents the esculentum alleles and 1, represents the hirsutum alleles.

tic interaction (at the 5% level) was observed. The gametes that were favored had either the $Pgi\text{-}1^1$ allele or the $Adh\text{-}2^1$ allele. Pollen grains bearing both alleles were selected against in the cold, an unexpected type of interaction.

DISCUSSION

In a previous study we showed that pollen grains of *L. hirsutum* are better adapted to complete fertilization at low temperatures than pollen of the cultivated tomato (ZAMIR, TANKSLEY and JONES 1981). The question naturally arises as to whether this response is due to maternal effects or to genes expressed by the haploid gametes. In the present study, pollen grains were harvested from a single F_1 hybrid between the two species. If we assume that maternal effects are identical for all the pollen grains, any selection response in the gamete population can only be attributed to genes expressed by the haploid gametophytes.

The isozyme markers cover regions of at least six of the 12 tomato chromosomes. The allelic frequencies of *Prx-1, Pgm-2, Got-2, Aps-1, Aps-2* and *Prx-4* did not vary significantly between the treatments. Skewing was observed in the frequencies of *Pgi-1, Est-4* and *Adh-2*. Gametes containing two independent hirsutum chromosome segments marked by $Pgi\text{-}1^1$, $Est\text{-}4^1$ and $Adh\text{-}2^1$ were significantly more successful in completing fertilization at low temperatures than gametes that had the esculentum alleles. This selective response demonstrates that genes expressed by the haploid pollen grains are responsible for differential fertilization ability at low temperatures. It further demonstrates that "cold responding" gene(s) are located on the two marked chromosome segments. Only three of the nine enzyme loci have shown a significant temperature response. This suggests that fertilization ability of pollen grains under the stress conditions is not determined by a large proportion of the haploid genome.

In nature, environmental conditions differ greatly from those encountered by plants in our experimental system. Our main consideration in determining the conditions of the experiment was to subject the plants to low temperatures that would permit selected gametes to complete fertilization. Under the light/time cycle conditions of the experiment, the rates of photosynthesis in tomato plants are negligible, particularly at low temperature (Ho 1976). The temperature conditions have an additional differential effect on carbon metabolism and translocation rates from the vegetative parts of the plant to the developing fruits (WALKER and Ho 1977). Tomato plants respond to low temperature by increasing the concentrations of abscisic acid, a plant hormone often associated with stress conditions (DAIE and CAMPBELL 1981). These examples demonstrate why fertilization ability of male gametes must be considered in respect to the state of the female plants and flowers. Yet we feel that these limitations do not interfere with the central issue of our investigation, which was to determine if the pollen genome is responsible for the differential fertilization ability at high and low temperature regimes.

Discussion of the BC_1 data produced by pollen of the interspecific hybrid would not be complete without considering possible abnormalities which are common in species hybrids. Differential zygotic lethality in progeny of the interspecific hybrid between *L. chilense* and *L. esculentum* has been reported by RICK (1963). In our experiment cultural conditions were identical for progeny of crosses from the two temperature regimes. This fact and the lack of heterogeneity between the three replications lead us to assume that the BC_1 populations were not subjected to a strong differential selection during embryogenesis and seed germination. Similar conclusions were reached in a report of controlled introgression of *Solanum pennellii* chromosomes to *L. esculentum* (RICK 1969).

In the introduction to this paper, we cited a number of studies which demonstrated that haploid gametophytic selection can modify quantitative characters of the sporophytic generation. The effect of pollen selection on the sporophytic generation may be of evolutionary significance, particularly if a portion of the genetic repertoire is expressed both by the sporophytic and gametophytic phases of the life cycle (MULCAHY 1979). Due to such an overlap in gene expression, gamete selection could have a corresponding effect on the sporophyte (TANKSLEY, ZAMIR and RICK 1981).

Our report demonstrates that the low temperature tolerance of *L. hirsutum* pollen grains is determined by genes expressed in the pollen genome. Two chromosomal regions of hirsutum that are linked to the enzymic genes *Pgi-1* and *Adh-2* are highly favored in crosses at low temperature. It remains to be determined if any low temperature tolerance parameters of tomato sporophytes are effected by the same chromosomal regions.

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